TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

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Application For Research Grant

OK

Date: August 22, 1955

1. Name of Investigator: Dr. Simon H. Wender

would like to confer

2. Title: Research Professor of Chemistry

3. Institution & Address: University of Oklahoma Research Institute
Office of the Executive Director
Norman, Oklahoma

4. Project or Subject: A qualitative and quantitative study of the individual polyphenol content of cigarette tobacco and of the smoke and "tars" resulting from cigarette smoking, and also to study the fate of these compounds in the animal respiratory system.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

See #10 for background information

The proposed research will involve:

(a) expanded qualitative and new quantitative studies of as many as possible of the individual polyphenolic compounds present in tobacco leaves;

(b) the actual isolation of as many as possible of pure, randomly labeled, polyphenolic compounds from radioactive tobacco (now available from Argonne National Laboratory);

(c) qualitative and quantitative studies of the individual polyphenols present in the tobacco of commercial cigarettes and then

(d) using individual, radioactive polyphenolic compounds known to be generally present in the cigarette (in non-radioactive condition, of course), in studies of the fate of these compounds in the smoke and "tars" resulting from the smoking of cigarettes; and

(e) studies on the fate of these compounds in the respiratory system of laboratory test animals.

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8. Facilities and Staff Available -- continued

Dr. Wender was born in Dalton, Georgia, September 4, 1913. He received the A.B. and M.S. degrees at Emory University, Georgia, in 1934 and 1935, respectively, and the Ph.D. in Agricultural Biochemistry (Major Professor, the late Dr. Ross A. Gortner) from the University of Minnesota in 1938. Member, Phi Beta Kappa, Sigma Xi, Phi Lambda Upsilon, Phi Sigma, Alpha Chi Sigma, American Chemical Society, (now chairman of its Oklahoma section) American Society of Biological Chemists, American Association for the Advancement of Science, Society for Experimental Biology and Medicine, Chemists Club of Central Oklahoma, and Oklahoma Academy of Science (Fellow).

Dr. Wender is representative of the University of Oklahoma to the Council of the Oak Ridge Institute of Nuclear Studies, and is a consultant to the Division of Biology and Medicine, Argonne National Laboratory.

Plant Physiologist, Dr. Lawrence M. Rohrbaugh, Professor of Plant Physiology at the University of Oklahoma, will be the consultant on plant physiology phases of the research.

Dr. Marvin Shetlar, Chief of the Medical Research Laboratory, V. A. Hospital, Oklahoma City, will cooperate on biochemical and physiological studies and act as a consultant to the project.

Other consultants on many specialties, including cancer specialists, will be available as necessary, at the School of Medicine, University of Oklahoma.

10. Additional Information -- continued

these polyphenols may be of some import in the planned analyses of tobacco smoke.

Reprints, where available, of some of the above and other pertinent articles are attached, as is a list of publications of Dr. Wender.

During the summer of 1954, while Dr. Wender was a research participant working with Dr. Norbet J. Scully and his staff at the Argonne National Laboratory, studies aimed at separating and identifying the many, individual polyphenolic compounds present in tobacco leaves were undertaken. Considerable initial progress was made, both on regular tobacco and also on tobacco plants grown in an atmosphere containing $C^{14}O_2$. This research has as its main purpose the acquisition of fundamental knowledge about the composition and metabolism of polyphenolic compounds in plants, and is still in active progress, with support from the U. S. Atomic Energy Commission.

During the past year, Dr. Wender and students at Oklahoma have further improved their chromatographic techniques, and have now successfully obtained more than 20 pure compounds from tobacco. No doubt, several of these compounds will be found to be identical on ultimate analysis, and two of these compounds have been previously reported to be present in tobacco. It appears, however, that at least 15 of these polyphenolic compounds are individuals not previously reported in tobacco. Fine progress is now being made on the difficult task of identification of these compounds.

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These isolation methods and this knowledge are now available for application to the tobacco grown in an atmosphere of $Cl^{4}O_2$ at the Argonne National Laboratory. Their application would make available many individual mm radioactive polyphenols for further studies.

A chemical method for the preparation of specifically labeled quercetin ~~xxxxxxx~~ (one of the compounds present in tobacco) is now being worked out at Oklahoma, and the Oklahoma group is now well along in the actual synthesis of this radioactive polyphenol. When completed, this will further supplement the potent array of tools for a possible investigation of the subject.

None of this work of the past year has as yet been published, but some of the isolation and identification studies are outlined in two M.S. theses just filed this month at the University of Oklahoma (M.S. theses of Mr. Edwin L. Murphy and Mr. Chao-Wen Hua Yang).

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Publications by Dr. Simon H. Wender

"The Isolation of Photosensitizing Agents from Buckwheat," J. Am. Chem. Soc. 65 1733 (1943).

"Preliminary Studies on the Separation of Ether-Insoluble Pigments from Tobacco," Trans. Ky. Acad. Sci. 11 36 (1943)

"Studies on Toxic Substances of Locoweeds," Texas Agric. Exp. Sta. Bull. No. 650 (1944).

"The Action of Photosensitizing Agents from Buckwheat," Amer. J. Vet. Res. 3 486 (1946)

"Studies on Flavone-Like Substances Isolated from Tobacco," Trans. Kyxx Ky. Acad. Sxx Sci. 12 10 (1947)

"A Method for the Separation of Certain Flavones Present in Tobacco," Proc. Okla. Acad. Sci. 27 95 (1947)

"The Use of Amberlite Resin in a Separation of Xanthine from Guanine," J. Am. Chem. Soc. ~~72~~ 70 3719 (1948)

"The Ultra violet Absorption Spectrum of Naringin by Use of the Beckman Model DU Spectrophotometer," Proc. Okla. Acad. Sci. 29 57 (1948)

"The Purification and Quantitative Estimation of Quercetin by Paper Partition Chromatography," Proc. Okla. Acad. Sci. 29 64 (1948)

"Chromatographic Adsorption Studies on Certain Flavones," Proc. Okla. Acad. Sci. 29 71 (1948)

X "Asparagine Content of Burley Tobacco After Long Storage," Bot. Gaz. 110 637 (1949)

"Paper Chromatography of Flavonoid Pigments," Science 109 287 (1949)

"Paper Chromatography of Flavonoid Pigments: II Separation and Quantitative Estimation of Rutin and Quercetin," Federation Proc. 8 298 (1949)

X indicates that reprints are on file in the Tobacco Industry Research Committee office.

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Source: https://www.industrydocuments.ucsf.edu/docs/hrll0000

X "The Isolation of Isoquercitrin from the Seed Pods of Cercis Canadensis," J. Am. Chem. Soc. 71 2658 (1949)

"Preliminary Studies on the Use of Amberlite Resins in Locoweed Investigations," Proc. Okla. Acad. Sci. 30 150 (1949)

"The Use of Metal Complexes in Identification of Flavonoid Pigments," Proc. Okla. Acad. Sci. 30 145 (1949)

"A Method for the Purification of Ethanol for Use in Ultraviolet Absorption Spectrophotometry," Proc. Okla. Acad. Sci. 30 149 (1949)

"The Calibration of Pipettes for Paper Partition Chromatography," Proc. Okla. Acad. Sci. 30 144 (1949)

"Use of Multiple Strips in One-Dimensional Paper Chromatography," Proc. Okla. Acad. Sci. 30 153 (1949)

X "The Isolation of Isoquercitrin from Air-Dried Tobacco," Arch. Biochem. 25 74 (1950)

"A Simplified Laboratory Experiment in Paper Partition Chromatography," J. Chem. Educ. 27 159 (1950)

X "The Quantitative Determination of Certain Flavonol-3-Glycosids by Paper Partition Chromatography," Analytical Chem. 22 709 (1950)

X "The Isolation of Quercitrin from Peanut Hulls," J. Am. Chem. Soc. 72 4177 (1950)

"The Isolation of a Flavonoid Glycoside from Gleditsia triacanthos," Proc. Okla. Acad. Sci. 31 137 (1950)

"Preliminary Studies on a Flavonoid Pigment from the Fruit of Plantanus occidentalis," Proc. Okla. Acad. Sci. 31 138 (1950)

"The Use of Ion Exchange Resins in the Isolation of Flavonoid Compounds from Okra," Proc. Okla. Acad. Sci. 31 140 (1950)

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"Preliminary Investigations on the Flavonoid Pigments of Prunes," Proc. Okla. Acad. Sci. 31 93 (1950)

"Chemical Studies on a Toxic Concentrate from the Big Bend Locoweed," Proc. Okla. Acad. Sci. 31 66 (1950)

"Methods for the Isolation of Quercitrin from Peanut Hulls," U. S. Patent 2,557,164 (1951)

X "The Use of Ion Exchange Resins in the Isolation of Flavonoid Compounds," Science 113 522 (1951)

X "The Use of N-bromosuccinimide and Pyridinium Bromide Perbromide for the Conversion of Flavanones into Flavones," J. Org. Chem. 16 930 (1951)

X "The Isolation of Morin on an Ion Exchange Resin," J. Am. Chem. Soc. 73 3340 (1951)

X "The 2,4-Dinitrophenylhydrazine Derivatives of Some Flavanones," J. Amer. Chem. Soc. 73 4023 (1951)

"Identification of Flavonoid Compounds by Filter Paper Chromatography," Anal. Chem. 23 1582 (1951)

"The Use of Filter Paper Pulp in the Separation of Certain Flavonoid Compounds," Proc. Okla. Acad.Sci. 32 99 (1951)

"Preliminary Studies on the Anthocyanins of Phytolacca americana on Amberlite IRC-50," Proc. Okla. Acad. Sci. 32 101 (1951)

X "Quantitative Paper Chromatography Studies on Flavonol Aglycones," Proc. Okla. Acad. Sci. 32 102 (1951)

"The Use of Ion Exchange Resins in the Decomposition of Lead Salts of Flavonoid Compounds," Proc. Okla. Acad. Sci. 32 100 (1951)

"A Convenient Laboratory Model Wet Grinder and Extractor," Anal. Chem. 24 767 (1952)

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Source: https://www.industrydocuments.ucsf.edu/docs/sbhl0000

X "Acid Dissociation Exponents of Rutin and Xanthorhamnin," J. Am. Chem. Soc. 74 143 (1952)

X "A New Method for the Isolation of Pure Quercitrin from Lemon Flavin," Arch. Biochem. and Biophys. 38 195 (1952)

X "The Isolation and Identification of Quercetin and Isoquercitrin from Grapes (Vitis vinifera)," J. Amer. Chem. Soc. 74 4372 (1952)

"The Isolation and Identification of Quercetin and Isoquercitrin from Black Currants (Ribes nigrum)," J. Amer. Chem. Soc. 74 4566 (1952)

X "The Synthesis of Isoquercitrin," J. Amer. Chem. Soc. 74 4606 (1952)

X "Adsorption Chromatography of Flavonoid Compounds," Anal. Chem. 24 1616 (1952)

X "The Isolation and Identification of Kaempferol and Quercetin from Strawberries (Fragaria chiloensis)," J. Amer. Chem. Soc. 74 5919 (1952)

"Studies on Flavonoids of Locoweed," Proc. Okla. Acad. Science 33 251 (1952)

"Further Fractionation of a Toxic Concentrate from Big Bend Locoweed," Proc. Okla. Acad. Science 33 253 (1952)

"The Isolation of a Flavonoid Substance from Watermelon," Proc. Okla. Acad. Science 33 247 (1952)

X "Isoquercitrin and Quercetin in Concord Grapes," Proc. Okla. Acad. Science, 33 250 (1952)

"Preliminary Investigations of the Possible Flavonol Content of the Pineapple," Proc. Okla. Acad. Science 33 249 (1952)

"Identification of Flavonoid Compounds by Filter Paper Chromatography. II Additional R_f Values and Color Tests," Anal. Chem. 25 503 (1953)

"Quercetin and Its Glycosides in Leaves of Vaccinium myrtillus," J. American Chem. Soc. 75 50 (1953)

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X "Isolation and Identification of Quercetin and Isoquercitrin from Apricots (Prunus armeniaca)" Arch. Biochem. and Biophys. 43 22 379 (1953)

X "An Improved Method of Analysis for Glycine Using Streptococcus Faecalis A.T.C.C. 6057," Arch. Biochem. and Biophys. 43 465 (1953)

X "Hydrolysis of Some Flavonoid Rhamnoglucosides to Flavonoid Glucosides," J. Amer. Chem. Soc. 75 2504 (1953)

"Some Factors Affecting the Nutritional Requirements of Streptococcus Faecalis A.T.C.C. 6057," J. Bact. 65 660 (1953)

"Separation and Determination of Pyridoxine, Pyridoxal, and Pyridoxamine by Paper Chromatography and Microbiological Assay," Arch. Biochem. and Biophys. 46 465 (1953)

X "Isolation and Identification of Quercetin and Some Quercetin Glycosides from Plums (Prunus salicina)," J. Amer. Chem. Soc. 75 4363 (1953)

X "Enzymatic Hydrolysis of Certain Flavonoid Glycosides," J. Amer. Chem. Soc. 76 1950 (1954)

"Ion Exchange Studies on the Flavonoid Fraction from Licorice Root", Proc. Okla. Acad. Sci. (1953) 34 163 (1953)

"Isolation of Flavonoid Compounds," U. S. Patent No. 2,681,097 (1954)

X "Amperometric Titrations of Some Flavonoid Compounds with ~~Rutin~~ Cupric Sulfate," J. Am. Chem. Soc. 77 162 (1955)

Patents Pending through the Atomic Energy Commission

"Separation of Flavonoid Compounds," A.E.C. Case No. S-4300 (S. N. 285,046)

"Improved Method for the Separation of Flavonoid Compounds, " A.E.C. Case S-10,133 (S. N. 323,505)

"Synthesis of an Aldohexoside of a Flavonol," A.E.C. Case S-10,134 (S. N. 339,928)

"A Method for the Conversion of Flavonoid Glucosides," A.E.C. Case No. S-10,724

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6. Budget Plan:

Salaries	$ 5,250.00
Expendable Supplies	1,250.00
Permanent Equipment	1,000.00
Overhead	1,170.00
Other (travel, transp., com.)	300.00
Total	$ 8,970.00

7. Anticipated Duration of Work: It is estimated that some three to five years will be necessary for satisfactory achievement of the major objectives of the projected research. Considerable progress, however, could be expected within one year.

8. Facilities and Staff Available:

Adequate research facilities for the proper prosecution of the investigation are available.

Staff available: Plant Biochemist, Dr. Simon H. Wender

The project will be supervised by Dr. Simon H. Wender, Research Professor of Chemistry, Department of Chemistry, University of Oklahoma
(see attached sheet)

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

In the past few years, by means of chromatographic adsorption, paper chromatographic, and ion exchange methods recently adapted to the polyphenolic flavonoid compounds in the laboratories of the University of Oklahoma, it has been found possible to effect the separation of flavonoid compounds from the tannins, anthocyanins, and other phenolic compounds present in the plant extracts and also to separate individual flavonoids from each other. (Anal. Chem. 22 709 (1950); 23 1582 (1951); 24 1616 (1952); and 25 508 (1953); Science 109 287 (1949) and 113 522 (1951). In addition, paper chromatography may be used for the qualitative and quantitative analyses of individual compounds in this group. By such techniques, Oklahoma ~~xxx~~ workers have been able to identify the polyphenolic flavonoid compound isoquercitrin in tobacco which had been stored for more than seven years (Arch. Biochem. 25 74 (1950). Work with anthocyanins and other phenolic compounds has indicated similar possibilities with them. Thus, chromatography, together with the extremely sensitive tool of radioactive polyphenols, now make the research proposed here feasible. Also many of these compounds to be studied have high melting points and readily sublime. Therefore,
(continued on attached sheet)

s/ Simon H. Wender
Signature
Director of Project

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s/ Lloyd E. Swearingen
Business Officer of the Institution

Executive Director - University of Oklahoma Research Institute, Norman, Oklahoma

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